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A Mendelian randomization study of the causal association between anxiety phenotypes and schizophrenia

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Schizophrenia shows a genetic correlation with both anxiety disorder and neuroticism, a trait strongly associated with anxiety. However, genetic correlations do not discern causality from genetic confounding. We therefore aimed to investigate whether anxiety-related phenotypes lie on the causal pathway to schizophrenia using Mendelian randomization (MR). Four MR methods, each with different assumptions regarding instrument validity, were used to investigate casual associations of anxiety and neuroticism related phenotypes on schizophrenia, and vice versa: inverse variance weighted (IVW), weighted median, weighted mode, and, when appropriate, MR Egger regression. MR provided evidence of a causal effect of neuroticism on schizophrenia (IVW odds ratio [OR]: 1.33, 95% confidence interval [CI]: 1.12–1.59), but only weak evidence of a causal effect of anxiety on schizophrenia (IVW OR: 1.10, 95% CI: 1.01–1.19). There was also evidence of a causal association from schizophrenia liability to anxiety disorder (IVW OR: 1.28, 95% CI: 1.18–1.39) and worry (IVW beta: 0.05, 95% CI: 0.03–0.07), but effect estimates from schizophrenia to neuroticism were inconsistent in the main analysis. The evidence of neuroticism increasing schizophrenia risk provided by our results supports future efforts to evaluate neuroticism- or anxiety-based therapies to prevent onset of psychotic disorders.

KEYWORDS
anxiety, Mendelian randomization, neuroticism, schizophrenia

1 | BACKGROUND

Schizophrenia is a heritable psychotic disorder characterized by positive (e.g., hallucinations and delusions) and negative (e.g., apathy and flattened affect) symptoms. It is associated with significant health, social and financial burden (Chong et al., 2016). Anxiety symptoms are prevalent among people with schizophrenia (Temmingh & Stein, 2015) with meta-analyses demonstrating that anxiety symptoms reach the threshold of disorder in an estimated 38% of patients (Achim et al., 2011). Anxiety disorders are also present in people with first episode psychosis (Michail & Birchwood, 2014) and those at high risk for psychosis (Fusar-Poli, Nelson, Valmaggia, Yung, & McGuire, 2014; Gajwani, Patterson, & Birchwood, 2013) and have been shown to precede psychosis onset (Welham, Isohanni, Jones, & McGrath, 2009), suggesting they do not occur only as a consequence of psychotic disorder onset or treatment.

Neuroticism is a personality trait that describes a dispositional tendency to become aroused quickly when stimulated and to be slow in inhibiting emotions. Individuals scoring highly on neuroticism experience negative emotional states, such as worry and guilt, particularly

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in response to threat or frustration (Ormel, Bastiaansen, et al., 2013). Neuroticism is strongly associated with common mental disorders such as anxiety and depression (Kotov, Gamez, Schmidt, & Watson, 2010; Lahey, 2009) and longitudinal studies have shown it to be associated with an increased risk of subsequent psychotic symptoms (Goodwin, Fergusson, & Horwood, 2003; Krabbendam et al., 2002) and schizophrenia (Lönnqvist et al., 2009; Van Os & Jones, 2001).

Schizophrenia shows moderate genetic correlation with anxiety disorder (Otowa et al., 2016; Purves et al., 2019), neuroticism, and the genetically distinguishable "worry" subtype of neuroticism (Nagel, Jansen, et al., 2018). Genetic risk for schizophrenia has also been shown to be associated with a higher risk of anxiety disorders in adolescence and adulthood (Jones et al., 2016; Richards et al., 2019), while genetic risk for neuroticism is associated with negative symptoms in adolescence (Jones et al., 2018).

It has been suggested that anxiety might be on the causal pathway to schizophrenia (Hall, 2017), although it is also possible that anxiety arises secondary to the expression of schizophrenia genetic liability (e.g., through poor social cognition skills, such as deficits in emotion processing [Germine et al., 2016]), or that the association between anxiety and schizophrenia is due to confounding, including genetic confounding. For example, a genetic variant influencing anxiety may be in linkage disequilibrium (LD) (i.e., non-randomly correlated) with a genetic variant influencing schizophrenia, or a genetic variant may independently influence both anxiety and schizophrenia (termed horizontal pleiotropy).

If strong evidence is found that anxiety has a causal effect on the development of schizophrenia, then this would highlight the need for a more proactive approach to treating anxiety, both to prevent onset of psychosis in those at higher-risk, and to prevent relapse in those with schizophrenia. However, as it is difficult to tease out causal effects from reverse causation or confounding using traditional epidemiological approaches, more robust methods are needed. Mendelian randomization (MR) uses genetic variants as instrumental variables to investigate causal relationships between modifiable risk factors and health outcomes (Davey Smith & Ebrahim, 2003; Lawlor, Harbord, Sterne, Timpson, & Davey Smith, 2008). The core assumptions of MR are i) the genetic instrumental variables must be associated with the risk factor of interest, ii) they share no common cause with the outcome (i.e., are independent of confounders), and iii) they only affect the outcome through the risk factor (the exclusion restriction assumption). If these assumptions are met, this approach can overcome issues of reverse causation and unmeasured confounding. Two-sample MR is an extension of MR that allows the instrument-exposure and instrument-outcome associations to be measured in two independent samples (Pierce & Burgess, 2013). An advantage of a two-sample approach is that it can be implemented using summary data from large scale genome-wide association studies (GWASs) (Burgess, Butterworth, & Thompson, 2013), providing an opportunity to substantially increase statistical power. We therefore aimed to examine whether anxiety or neuroticism have a causal effect on schizophrenia using a two-sample MR study design.

2 | METHODS

2.1 | Genetic instrument data sources

2.1.1 | Anxiety

Genetic instruments for anxiety were taken from the 2019 lifetime anxiety disorder GWAS by Purves et al. (2019) who reported 5 independent loci that were genome-wide significantly (p value $\leq 5 \times 10^{-8}$) associated with lifetime anxiety disorder within UK Biobank (Western European ancestry; 25,453 cases, 58,113 controls; single nucleotide polymorphism [SNP]-based heritability on observed scale = 0.12). Lifetime anxiety disorder was defined by a self-reported lifetime professional diagnosis of one of the five core anxiety disorders (generalized anxiety disorder, social phobia, panic disorder, agoraphobia or specific phobia) or meeting criteria for a likely lifetime diagnosis of DSM-IV generalized anxiety disorder based on anxiety questions from the Composite International Diagnostic Interview Short-form questionnaire (Purves et al., 2019). Following a meta-analysis of the UK Biobank GWAS and GWASs from two additional studies (all European ancestry; total sample of 31,977 cases, 82,114 controls), the study reported 2 genome-wide significant SNPs. As one of the genome-wide significant SNPs (chromosome 5: rs7723509) had palindromic alleles with intermediate allele frequencies, this SNP was not taken forward in the analysis. The remaining genome-wide significant SNP (chromosome 9: rs10959577) was used within a single SNP, two-sample MR analysis (see below). Full GWAS summary statistics were obtained from the corresponding authors of the GWAS manuscript (Purves et al., 2019).

2.1.2 | Neuroticism

Genetic instruments for neuroticism were taken from a recent GWAS by Luciano et al. (2018) who reported 116 independent (R^2 < .1 within a 500 kb window) SNPs that were genome-wide significantly associated with a total neuroticism score based on the 12-item Eysenck Personality Questionnaire Revised Short Form (EPQ-R-S) within UK Biobank (white British ancestry; n = 329,821 participants; SNP-based heritability = 0.11). Full GWAS summary statistics are available from: http://www.ccace.ed.ac.uk/node/335.

2.1.3 | Depressed affect and worry

Genetic instruments for 2 sub-clusters of neuroticism (depressed affect and worry) (Nagel, Watanabe, Stringer, Posthuma, & van der Sluis, 2018) were taken from Nagel, Jansen, et al. (2018) who performed a GWAS in UK Biobank using 4 EPQ-R-S items relating to a depressed affect sub-cluster (European ancestry; n = 357,957 participants; SNP-based heritability = 0.09) and 4 EPQ-R-S items relating to a worry sub-cluster (European ancestry; n = 348,219 participants; SNP-based heritability = 0.09). Following functional mapping of genome-wide significance SNPs, the study reported 75 independent

(R^2 < .1) lead SNPs for depressed affect and 73 independent (R^2 < .1) lead SNPs for worry. Full GWAS summary statistics are available from: https://ctg.cncr.nl/software/summary_statistics.

2.1.4 | Schizophrenia

Genetic instruments for schizophrenia were taken from the 2014 Psychiatric Genomics Consortium GWAS which reported 128 independent (R^2 < .1 within a 500 kb window) SNPs that were genome-wide significantly associated with schizophrenia case/control status after a meta-analysis of 49 case/control GWASs (European ancestry; 33,640 cases, 43,456 controls; SNP-based heritability on observed scale = 0.45) (Schizophrenia Working Group of the Psychiatric Genomics Consortium, 2014). Full GWAS summary statistics are available from: https://www.med.unc.edu/pgc/results-and-downloads/.

2.2 | Bidirectional, two-sample MR

To investigate the direction of causality between schizophrenia and the anxiety-related phenotypes, a bidirectional two-sample MR approach was used where the anxiety-related phenotypes were treated as the exposures in one set of analyses and schizophrenia was treated as the exposure in another set of analyses. GWAS summary statistics relating to genome-wide significant SNPs associated with anxiety disorder, neuroticism (as well as depressed affect and worry sub-clusters) and schizophrenia were used as exposure instruments.

SNPs were included in the analysis if they had a minor allele frequency (MAF) ≥ 0.05. SNP alleles, phenotype association effect sizes, standard errors and *p* values for each exposure genetic instrument were extracted from the corresponding exposure GWASs. To ensure that the SNPs were independent, SNPs were pruned for LD using the - -r2 command in PLINK (v1.9) (Chang et al., 2015; Purcell et al., 2007) with 1000genomes (phase 1 version 3) as a reference panel. SNPs were deemed as being in LD if they were correlated at R^2 > .01 within a 10,000 kb window. The SNP with the largest GWAS standard error from each correlated SNP pair was excluded from the analyses.

Following LD pruning, exposure SNP information (mainly SNP-phenotype effect sizes, standard errors and *p* values, effect and alternative alleles and effect allele frequency) was harmonized with the corresponding SNP information, if available, from the outcome GWAS summary statistics using the 2sampleMR MR-Base R package (Hemani et al., 2018). During harmonization, SNPs were excluded based on allele differences, strand differences and if palindromic SNPs had a MAF > 0.42. See Table S1 for the number of SNPs retained for each analysis.

All MR analyses were carried out using the 2sampleMR MR-Base R package. For the single SNP analysis, a ratio estimate was calculated by dividing the SNP-schizophrenia effect estimate by the SNP-anxiety effect estimate with standard errors derived using the first term from a delta method expansion for the ratio estimate (Thomas, Lawlor, & Thompson, 2007). For multi-SNP analyses, four regression-based methods were used to pool and assess causal estimates between anxiety disorder, neuroticism phenotypes and schizophrenia. These included inverse variance weighted (IVW), weighted median, weighted mode, and MR Egger regression methods. Briefly, the IVW method is equivalent to a weighted linear regression of SNP-outcome associations on SNP-exposure associations with the assumption that all SNPs are valid instruments, that is, there is no directional pleiotropy (Burgess et al., 2013; Lawlor et al., 2008). Because of this assumption, the intercept of the IVW regression is constrained to zero (i.e., if there is no effect of the SNP on the exposure, there will be no effect of the SNP on the outcome). The weighted median method estimates the causal effect from the median of the weighted empirical density function of SNP-outcome/SNP-exposure ratio estimates (Bowden, Davey Smith, Haycock, & Burgess, 2016). This method thus allows up to 50% of the information in the analysis to come from invalid SNPs. The weighted mode method estimates the causal effect from the mode of the weighted empirical density function of SNP-outcome/SNP-exposure ratio estimates and assumes that the weights associated with valid instruments are the largest among all subsets of instruments (the ZEro Modal Pleiotropy Assumption) (Hartwig, Davey Smith, & Bowden, 2017). The MR Egger regression method is an expansion of the IVW method which does not assume that all instruments are valid and thus does not constrain the regression intercept to zero (Bowden, Davey Smith, & Burgess, 2015). The method therefore provides a causal estimate that takes pleiotropic effects into account with the intercept giving an estimate of the average pleiotropic effect (i.e., effect of the SNP on the outcome when there is no effect of the SNP on the exposure). The MR Egger method gives a valid causal estimate if the SNP-exposure associations are not correlated to the direct effects of the genetic variants on the outcome (i.e., pleiotropic effects). This is termed the Instrument Strength Independent of Direct Effect (InSIDE) assumption (Bowden et al., 2015).

2.3 | Assessing instrument strength and heterogeneity

Weak instrument bias within the IVW analyses was quantified using the mean F statistic ($\bar{F}$) (Bowden, Del Greco, et al., 2016) with $\bar{F}$ > 10 indicating that the IVW analysis does not suffer substantially from weak instrument bias. The degree of violation of the IVW and MR-Egger assumption that the SNP-exposure association is measured without error (the "NO Measurement Error" [NOME] assumption) was assessed using $\bar{F}$ minus 1 divided by $\bar{F}$ ($(\bar{F}-1)/\bar{F}$) (IVW) and I^2_{GX} statistic (MR Egger) (Bowden, Del Greco, et al., 2016). These statistics range from 0 to 1, with values close to 1 indicating minimal attenuation in the effect estimate due to violation of the NOME assumption (Bowden et al., 2017; Bowden, Del Greco, et al., 2016). In situations where I^2_{GX} was relatively large (here we have defined this as >70%), simulation extrapolation (SIMEX) was also used as a method of bias adjustment for the MR Egger estimate in the presence of violation of the NOME assumption.

Presence of heterogeneity between individual SNP-outcome on SNP-exposure effect estimates was assessed using Cochran's (IVW)

and Rücker's (MR Egger) *Q* tests (Bowden et al., 2017; Del Greco, Minelli, Sheehan, & Thompson, 2015).

2.4 | Exclusion of instruments in linkage disequilibrium between exposure and outcome

As a sensitivity analysis to minimize potential violation of the second (instruments are independent of confounders) and third (instruments only affect the outcome through the exposure) core MR assumptions, two-sample MR analyses were repeated after excluding pairs of SNPs that were in LD between each exposure/outcome instrument set. Although these shared loci (that are strongly associated with both the exposures and outcomes in our analyses) may reflect truly causal loci, they may also index risk for something (such as a behavior) that increases risk of both anxiety and schizophrenia, or they might reflect horizontal pleiotropy (influencing the two phenotypes through independent pathways) or confounding by LD (associated with phenotypes through LD) (-Figure S1). These violations would in turn bias the bidirectional analyses.

LD between SNP instruments for anxiety/neuroticism phenotypes and schizophrenia was assessed using the methods described previously. Any SNP pair that was correlated at $R^2 > .01$ within a 10,000 kb window between the anxiety/neuroticism phenotype instruments and schizophrenia instruments were excluded from the sensitivity analysis (Tables S2–S5).

3 | RESULTS

3.1 | Instrument strength and heterogeneity

All $\bar{F}$ statistics were >10 indicating that weak instrument bias was not affecting the IVW analyses (Table S6). With regards to violation of the NOME assumption, $((\bar{F}-1)/\bar{F})$ and I^2_{GX} statistics indicated that measurement error in the SNP-exposure associations was not substantially attenuating the neuroticism to schizophrenia effect estimate ($(\bar{F}-1)/\bar{F} = 0.97$, $I^2_{GX} = 0.72$). However, all other I^2_{GX} statistics were low (I^2_{GX} range = 0.00–0.19) indicating that MR Egger effect estimates were potentially affected by violation of the NOME assumption (Table S6). We therefore have only presented the MR Egger estimates when investigating neuroticism as an exposure but present results of all other MR methods that are more robust to violations of NOME for other exposures.

There was strong evidence of heterogeneity in causal effect sizes across all analyses with the exception of the analysis investigating anxiety disorder as the exposure and schizophrenia as the outcome (Cochran's $Q = 2.74$; p value = .60; Table S6). Sensitivity plots depicting individual SNP effect estimates, "leave one out" analyses and instrument precision for each of the analyses are presented in Figures S6–S14.

3.2 | Anxiety as exposure

Table 1 and Figure S2a display the MR results of the association between genetically increased odds of having an anxiety disorder and schizophrenia. Across all MR approaches, estimated effect sizes were in the direction of a causal association between anxiety disorder and schizophrenia; however, the confidence intervals (CIs) often included protective effects (single SNP method odds ratio [OR]: 1.19, 95% CI: 0.93–1.52; IVW OR: 1.10, 95% CI: 1.01–1.19; weighted median OR: 1.05, 95% CI: 0.94, 1.16; weighted mode OR: 1.04, 95% CI: 0.90–1.21). Results were similar in the sensitivity analyses omitting instruments that were highly correlated between anxiety disorder and schizophrenia (i.e., potential shared loci between the exposure and the outcome) (Table 1 and Figure S2b).

3.3 | Neuroticism phenotypes as exposures

When investigating the association between genetically elevated levels of neuroticism and schizophrenia, all MR approaches showed

TABLE 1 Odds ratios of schizophrenia per increased odds of anxiety disorder as estimated by multiple Mendelian randomization methods

Exposure	Outcome	MR method	No. SNPs	OR (95% CI)	*p*
Following harmonization with outcome data					
Anxiety	Schizophrenia	Ratio estimate	1	1.19 (0.93, 1.52)	.164
		IVW	5	1.10 (1.01, 1.19)	.028
		Weighted median		1.05 (0.94, 1.16)	.372
		Weighted mode		1.04 (0.90, 1.21)	.624
Following harmonization with outcome data and removal of shared loci[a]					
Anxiety	Schizophrenia	IVW	4	1.11 (1.01, 1.21)	.027
		Weighted median		1.09 (0.98, 1.21)	.129
		Weighted mode		1.05 (0.89, 1.23)	.610

Note: MR Egger analyses were not performed due to large violation of the NOME assumption.
Abbreviations: 95% CI, 95% confidence interval; IVW, inverse variance weighted; MR, Mendelian randomization; No. SNPs, number of single nucleotide polymorphism used in the analysis as instruments; OR, odds ratio.
[a]Shared loci were defined as correlated anxiety and schizophrenia instruments ($R^2 > .01$ within a 10,000 kb window).

evidence that neuroticism increased the odds of schizophrenia (IVW OR: 1.33, 95% CI: 1.12–1.59; weighted median OR: 1.34, 95% CI: 1.16, 1.55; weighted mode OR: 1.43, 95% CI: 1.06–1.93; MR Egger OR: 2.06, 95% CI: 0.37, 11.53) (Table 2 and Figure S3a). The MR Egger regression intercept provided little evidence of directional horizontal pleiotropy (MR Egger intercept OR: 0.99, 95% CI: 0.93, 1.05). Estimates using MR Egger with SIMEX were consistent with MR Egger results without adjusting for bias induced by violation of the NOME assumption, though 95% CIs were wider (MR Egger OR: 5.32, 95% CI: 0.30, 93.59; MR Egger intercept OR: 0.95, 95% CI: 0.86, 1.05).

Sensitivity plots evaluating individual SNP effect estimates and leave-one-out analyses showed that no individual SNPs were driving the associations, and symmetry within the funnel plot evaluating instrument precision indicated little evidence of directional pleiotropy (Figure S7).

Similar results were observed when investigating the effects of genetically elevated levels of the neuroticism sub-clusters, depressed affect and worry, although evidence from the weighted mode analysis was weaker (Table 2, Figure S3b,c).

Results were similar to the primary analyses in the sensitivity analyses omitting instruments that were highly correlated between the neuroticism phenotypes and schizophrenia (Table 2, Figure S3d–f), however, the evidence of a causal effect of depressed affect and worry on schizophrenia substantially weakened.

3.4 | Schizophrenia as exposure

Tables 3 and 4 and Figures S4 and S5 display the MR results of the association between genetically increased odds of having schizophrenia and anxiety and neuroticism phenotypes. There was evidence, with consistent effect sizes across MR methods, of a causal association between schizophrenia liability and anxiety disorder (IVW OR: 1.28, 95% CI: 1.18–1.39; weighted median OR: 1.21, 95% CI: 1.10, 1.34), although evidence was weaker when using the weighted mode method (OR: 1.18, 95% CI: 0.94–1.49). Results were similar in sensitivity analyses omitting instruments that were highly correlated between anxiety disorder and schizophrenia (Table 3, Figure S4b). No individual SNPs were driving this association and symmetry within the funnel plot indicated little evidence of directional pleiotropy (Figure S10).

The strongest evidence of a causal association from schizophrenia liability to neuroticism was observed when using the IVW MR method (beta: 0.05, 95% CI: 0.01–0.09); however, there was little evidence observed when using the other MR methods with inconsistencies between direction of effect (Table 4, Figure S5a).

Similar to the association between schizophrenia and anxiety disorder, there was however, more consistent evidence of an effect of genetic liability for schizophrenia on levels of worry with strong evidence presented from the IVW and weighted median analyses (IVW beta: 0.05, 95% CI: 0.03–0.07; weighted median beta: 0.04, 95% CI: 0.02–0.05; weighted mode beta: 0.04, 95% CI: 0.00–0.07) (Table 4, Figure S5c), but not on depressed affect (Table 4, Figure S5a,b). No individual SNPs were driving this association, however there was some asymmetry within the funnel plot indicating evidence of directional pleiotropy (Figure S14).

In sensitivity analyses omitting loci correlated between neuroticism and schizophrenia phenotypes, results were similar to primary analyses with no strong evidence of effect of higher genetic liability to schizophrenia leading to changes in levels of neuroticism or depressed affect, though the directions of the effect estimates were now consistent, but strong evidence that genetic liability to schizophrenia is associated to higher levels of worry (Table 4, Figure S5d–f).

4 | DISCUSSION

The results of this two-sample MR study provide evidence of an association between schizophrenia and anxiety phenotypes as well as an association between neuroticism and schizophrenia.

Although anxiety has long been reported as a common feature of the schizophrenia prodrome (Docherty, Van Kammen, Siris, & Marder, 1978; Fusar-Poli et al., 2014; Tien & Eaton, 1992; Turnbull & Bebbington, 2001), using genetic instruments to proxy anxiety disorder, we found only weak evidence that increased odds of having anxiety increases risk of schizophrenia. The majority of the MR approaches we used however, indicated that a higher neuroticism score increases odds of schizophrenia. This result is in agreement with longitudinal studies that report an association between higher levels of neuroticism and increased risk of development of psychotic symptoms (Goodwin et al., 2003; Krabbendam et al., 2002) and schizophrenia (Lönnqvist et al., 2009; Van Os & Jones, 2001), as well as a previous MR of neuroticism and schizophrenia that used a generalized summary-data-based MR [GSMR] approach (Nagel, Jansen, et al., 2018). In contrast to the other methods, the MR Egger approach showed little evidence of association between neuroticism and schizophrenia. However, the power to detect causal effects using MR Egger, as well as the SIMEX bias adjustment method, is very sensitive to the amount of violation in the NOME assumption which is potentially still too large in the current study (Bowden, Del Greco, et al., 2016).

The conceptual understanding of the relationship between neuroticism and anxiety symptoms or disorder is not well understood. Theoretical models positing either that neuroticism is a separate construct that acts as a risk factor for anxiety disorders, or that neuroticism and anxiety symptoms/disorder lie on different parts of a spectrum or continuum are both partly supported by empirical evidence (Ormel, Jeronimus, et al., 2013). The difficulty in teasing apart neuroticism from anxiety is further complicated by the substantial overlap in questions used to measure these phenotypes, and the strong association between neuroticism and anxiety disorder in cross-sectional studies (Cohen's d >1.9 for most anxiety disorders) (Kotov et al., 2010). The findings from our neuroticism MR may therefore be consistent with anxiety having a causal effect on schizophrenia, particularly in light of the fact that the neuroticism instruments were

TABLE 2 Odds ratios of schizophrenia per unit increase in neuroticism phenotype score as estimated by multiple Mendelian randomization methods

Exposure	Outcome	MR method	No. SNPs	OR (95% CI)	*p*
Following harmonization with outcome data					
Neuroticism	Schizophrenia	IVW	71	1.33 (1.12, 1.59)	.001
		Weighted median		1.34 (1.16, 1.55)	$6.17e^{-05}$
		Weighted mode		1.43 (1.06, 1.93)	.023
		MR Egger slope		2.06 (0.37, 11.53)	.416
		MR Egger intercept		0.99 (0.99, 1.05)	.623
Depressed affect sub-cluster		IVW	54	1.54 (0.96, 2.46)	.073
		Weighted median		1.62 (1.11, 2.36)	.012
		Weighted mode		2.03 (0.90, 4.57)	.094
		MR Egger slope[a]		—	—
		MR Egger intercept[a]		—	—
Worry sub-cluster		IVW	57	2.54 (1.60, 4.03)	$7.11e^{-05}$
		Weighted median		1.57 (1.11, 2.23)	.011
		Weighted mode		1.26 (0.65, 2.44)	.494
		MR Egger slope[a]		—	—
		MR Egger intercept[a]		—	—
Following harmonization with outcome data and removal of shared loci[b]					
Neuroticism	Schizophrenia	IVW	50	1.30 (1.08, 1.56)	.006
		Weighted median		1.36 (1.14, 1.63)	.001
		Weighted mode		1.52 (1.09, 2.13)	.016
		MR Egger slope		0.93 (0.17, 4.94)	.929
		MR Egger intercept		1.01 (0.95, 1.07)	.694
Depressed affect sub-cluster		IVW	34	1.12 (0.65, 1.94)	.680
		Weighted median		1.52 (0.96, 2.40)	.076
		Weighted mode		2.11 (0.97, 4.60)	.070
		MR Egger slope[a]		—	—
		MR Egger intercept[a]		—	—
Worry sub-cluster		IVW	36	1.22 (0.78, 1.91)	.392
		Weighted median		1.22 (0.82, 1.83)	.332
		Weighted mode		1.13 (0.51, 2.54)	.766
		MR Egger slope[a]		—	—
		MR Egger intercept[a]		—	—

Abbreviations: 95% CI, 95% confidence interval; IVW, inverse variance weighted; MR, Mendelian randomization; No. SNPs, number of single nucleotide polymorphism used in the analysis as instruments; OR, odds ratio.

[a]MR Egger analyses not performed due to large violation of the NOME assumption.

[b]Shared loci were defined as correlated neuroticism phenotype and schizophrenia instruments (R^2 > .01 within a 10,000 kb window).

taken from a substantially larger GWAS than the anxiety disorder one. Future investigations utilizing joint analysis approaches, such as genomic structural equation modeling (Grotzinger et al., 2019) and multitrait-based conditional and joint analysis (Zhu et al., 2018), may be fruitful in shedding light on the shared and specific genetic architecture of these phenotypes once anxiety GWAS sample sizes increase.

We also found evidence that increased genetic liability to schizophrenia leads to higher levels of anxiety and the neuroticism subcluster relating to worry, a core feature of anxiety. Similar findings have been reported in our studies using polygenic scores for schizophrenia where genetic liability for the disorder is modeled using scores based on many risk-increasing SNPs, each with small effect. These previous studies showed that, within the general population, a higher genetic liability to schizophrenia is associated with anxiety disorder and with a latent construct of anxiety in adolescence (Jones et al., 2016; Jones et al., 2018), and with anxiety disorders, most strongly with GAD and panic disorder, in adulthood (Richards et al., 2019).

Together, these results imply that while neuroticism may confer a casual effect on risk of developing schizophrenia, higher neuroticism

TABLE 3 Odds ratios of anxiety disorder per increase in odds ratios of schizophrenia as estimated by multiple Mendelian randomization methods

Exposure	Outcome	MR method	No. SNPs	OR (95% CI)[a]	*p*
Following harmonization with outcome data					
Schizophrenia	Anxiety	IVW	84	1.28 (1.18, 1.39)	$6.15e^{-09}$
		Weighted median		1.21 (1.10, 1.34)	$1.48e^{-04}$
		Weighted mode		1.18 (0.94, 1.49)	.156
Following harmonization with outcome data and removal of shared loci[a]					
Schizophrenia	Anxiety	IVW	83	1.27 (1.17, 1.38)	$1.23e^{-08}$
		Weighted median		1.21 (1.10, 1.34)	$1.39e^{-04}$
		Weighted mode		1.19 (0.94, 1.50)	.147

Note: MR Egger analyses were not performed due to large violation of the NOME assumption.
Abbreviations: 95% CI, 95% confidence interval; IVW, inverse variance weighted; MR, Mendelian randomization; No. SNPs, number of single nucleotide polymorphism used in the analysis as instruments; OR, odds ratio.
[a]Shared loci were defined as correlated anxiety and schizophrenia instruments (R^2 > .01 within a 10,000 kb window).

TABLE 4 Change in neuroticism phenotype score per increase in odds ratios of schizophrenia as estimated by multiple Mendelian randomization methods

Exposure	Outcome	MR method	No. SNPs	Beta (95% CI)	*p*
Following harmonization with outcome data					
Schizophrenia	Neuroticism	IVW	82	0.05 (0.01, 0.09)	.009
		Weighted median		0.01 (−0.02, 0.04)	.414
		Weighted mode		−0.02 (−0.10, 0.06)	.679
	Depressed affect sub-cluster	IVW	82	0.01 (0.00, 0.03)	.077
		Weighted median		−0.01 (−0.02, 0.01)	.472
		Weighted mode		−0.02 (−0.06, 0.03)	.457
	Worry sub-cluster	IVW	82	0.05 (0.03, 0.07)	$3.14e^{-08}$
		Weighted median		0.04 (0.02, 0.05)	$6.66e^{-07}$
		Weighted mode		0.04 (−0.0004, 0.07)	.056
Following harmonization with outcome data and removal of shared loci[a]					
Schizophrenia	Neuroticism	IVW	58	0.04 (0.003, 0.08)	.034
		Weighted median		0.05 (0.01, 0.09)	.007
		Weighted mode		0.10 (−0.02, 0.21)	.109
	Depressed affect sub-cluster	IVW	61	0.01 (−0.01, 0.02)	.299
		Weighted median		0.01 (−0.01, 0.03)	.184
		Weighted mode		0.05 (−0.003, 0.11)	.069
	Worry sub-cluster	IVW	65	0.04 (0.02, 0.06)	$4.91e^{-06}$
		Weighted median		0.04 (0.02, 0.05)	$1.93e^{-06}$
		Weighted mode		0.04 (−0.001, 0.07)	.063

Note: MR Egger analyses were not performed due to large violation of the NOME assumption.
Abbreviations: 95% CI, 95% confidence interval; IVW, inverse variance weighted; MR, Mendelian randomization; No. SNPs, number of single nucleotide polymorphism used in the analysis as instruments; OR, odds ratio.
[a]Shared loci were defined as correlated neuroticism phenotype and schizophrenia instruments (R^2 > .01 within a 10,000 kb window).

scores and anxiety are also more likely to occur as a manifestation of schizophrenia liability, or secondary to the disorder. For example, it is difficult to envisage anyone hearing abusive voices or believing that others are trying to harm them without having some symptoms of anxiety in relation to these experiences.

There is some evidence that psychological treatments developed to address neuroticism have efficacy in treating anxiety disorders (Barlow et al., 2017). There is likely to be a large overlap in the cognitive-behavioral models underlying the treatment of neuroticism with those for specific anxiety disorders and targeting anxiety

symptoms also falls within the remit of cognitive-behavioral therapy for psychosis (Morrison, 2017). Therapies targeting neuroticism more explicitly have not yet been evaluated in prevention of psychosis, but based on our findings, might hold some promise.

High levels of anxiety in people with schizophrenia are associated with greater hallucinations, withdrawal, depression, hopelessness, and poorer function (Lysaker & Salyers, 2007). Therefore, while the likely benefit of targeting the treatment of neuroticism or anxiety to prevent transition to psychosis in people at clinical high-risk is unclear, psychological (Wykes, Steel, Everitt, & Tarrier, 2008) and pharmacological (Temmingh & Stein, 2015) therapies for anxiety may be useful not only in alleviating anxiety symptoms but also potentially in improving prognosis (Braga, Petrides, & Figueira, 2004) and quality of life (Braga, Mendlowicz, Marrocos, & Figueira, 2005) in people with a psychotic disorder.

Although we have used a causal inference design to assess the relationships between anxiety, neuroticism and schizophrenia, there are a number of limitations with our study. The first assumption of MR is that the genetic instrument must be strongly associated with the exposure (Lawlor et al., 2008). We attempted to satisfy this assumption by using genetic variants associated with our phenotypes at genome-wide significance. However, the instruments explain very little of the variance of these, typically polygenic, phenotypes. For example, genome-wide significant SNPs explain ~3% of variance in schizophrenia case–control status as compared to ~15% explained by SNPs meeting $p < .05$ (Schizophrenia Working Group of the Psychiatric Genomics Consortium, 2014). The variance explained by genome-wide significant SNPs was unfortunately not reported by the anxiety and neuroticism GWASs, however, SNPs meeting $p < .05$ explained only ~0.4% of variance in anxiety disorder case–control status (Purves et al., 2019) and ~3% of variance in the neuroticism score (Luciano et al., 2018). This means that the analyses, especially from anxiety to schizophrenia, may be subject to weak instrument bias which biases estimated effects toward the null (Pierce & Burgess, 2013), although our $\bar{F}$ statistics for all instruments suggest that our IVW results were not substantially affected by weak instrument bias. Nevertheless, it would be important to repeat these analyses using instruments detected in larger, and therefore better powered, GWASs once data from such studies become available.

We also observed substantial heterogeneity between causal effect estimates within the majority of analyses. Heterogeneity in effect estimates may be due to violation of the modeling assumptions of two-sample MR (e.g., that the exposure and outcome samples are homogenous) or due to presence of horizontal pleiotropy. Although the low I^2_{GX} prevented us from formally testing for pleiotropic effects across the majority of analyses, we attempted to minimize pleiotropic effects and confounding by using sensitivity analyses omitting shared loci between exposure and outcome. It is possible that these shared loci represent genetic liability to general psychopathology, commonly termed the *p* factor (Caspi et al., 2014), which may confound the true causal associations between schizophrenia and anxiety. However, if this were the case, we would expect removal of shared loci to weaken results in all analyses, which was not observed. Nevertheless, this approach did not improve our heterogeneity statistics and may have been limited by the use of the 1,000 genomes project phase 1 as an LD reference panel as opposed to a larger, more up to date panel such as that developed by the Haplotype Reference Consortium (McCarthy et al., 2016). We also tried to minimize heterogeneity between our samples by using SNP-effect estimates from samples with European ancestry. Despite this, other selection biases (e.g., using case–control samples vs. general population samples) may have reduced the level of homogeneity between our exposure and outcome samples.

Together, the low levels of variance explained by the instruments and presence of effect heterogeneity makes it difficult to be confident in interpreting the observed bidirectional relationship between these complex traits, where the underlying biological mechanisms that the instruments are proxying are poorly understood. Methods aimed at identifying and utilizing homogenous sub-groups of instruments to proxy distinct causal mechanisms, as they develop (Burgess, Foley, Allara, Staley, & Howson, 2020), will therefore be very useful in the future when investigating these multifactorial phenotypes.

Finally, it is apparent that the conceptual difference between neuroticism and anxiety is not clear with competing models presented throughout the literature (Ormel, Jeronimus, et al., 2013), while it is also unclear the extent to which measures used in GWASs of these phenotypes reflect separate or overlapping constructs. Therefore, as larger samples of more specific or more accurately measured phenotypes become available for GWASs, these should make it easier to tease out causal mechanisms that could be effectively targeted for interventions.

In conclusion, while there is evidence that schizophrenia liability increases anxiety, some evidence of neuroticism increasing schizophrenia risk supports further efforts to evaluate neuroticism- or anxiety-based therapies to prevent onset of psychotic disorders. As MR effect estimates represent lifetime risk, and should not be interpreted literally as the expected outcome of a clinical intervention, future efforts should focus on triangulation of results from two-sample MR with other study designs to improve our knowledge of causal pathways in psychosis etiology (Lawlor, Tilling, & Davey Smith, 2016).

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CONFLICT OF INTEREST

All authors declare that there are no conflicts of interest in relation to the subject of this study.

AUTHOR CONTRIBUTIONS

Hannah J. Jones, **Michael C. O'Donovan**, **Michael J. Owen**, **James T. R. Walters**, and **Stanley Zammit:** Conceived the project. **Hannah J. Jones:** Performed analyses. **George Davey Smith** and **Sarah**

J. Lewis: Provided statistical supervision. **Hannah J. Jones**, **David Martin** and **Stanley Zammit:** Wrote the draft manuscript, with subsequent revisions based on involvement from all listed authors.

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